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## Congenital Toxoplasmosis in a Indo-Pacific Bottlenose Dolphin (*Tursiops aduncus*)

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ABSTRACT: Toxoplasma gondii was identified in tissues of a stillborn late-term fetus from an Indo-Pacific bottlenose dolphin (Tursiops aduncus). Myocardial necrosis and nonsuppurative inflammation in the heart and nonsuppurative necrotizing encephalitis were associated with tachyzoites and tissue cysts. The diagnosis was confirmed by immuno-histochemical staining with anti-T. gondii-specific polyclonal rabbit serum.

There are several reports of Toxoplasma gondii-associated disease in sea mammals (Van Pelt and Dieterich, 1973; Migaki et al., 1977, 1990; Holshuh et al., 1985; Mikaelian et al., 2000). The first report of toxoplasmosis in a dolphin was from Brazil (Bandoli and Oliveira, 1977). Cruickshank et al. (1990) and Inskeep et al. (1990) reported toxoplasmosis in Atlantic bottlenose dolphins (Tursiops truncatus) from North America, and Domingo et al. (1992) reported T. gondii infection from striped dolphins (Stenella coeruleoalba) from Spain. These reports were based on histologic evaluation of dead animals. Cole et al. (2000) isolated several strains of T. gondii from the brains of California sea lions (Zalophus californianus), and Miller, Crosbie et al. (2001) and Miller, Sverlow et al. (2001) isolated T. gondii from a Pacific harbor seal (Phoca vitulina richardsi), also from California. In addition, encephalitis associated with the related protozoon Sarcocystis neurona has been diagnosed in 3 sea otters (Enhydra lutris) (Rosonke et al., 1999; Lindsay et al., 2000; Miller, Crosbie et al., 2001) and 7 Pacific harbor seals (Lapointe et al., 1998). Dual S. neurona and T. gondii infections have been confirmed in a sea otter (Lindsay et al., 2001) and a Pacific harbor seal (Miller, Sverlow et al., 2001). Here, we report a transplacentally transmitted T. gondii infection in a stillborn Indo-Pacific bottlenose dolphin ( $Tursiops\ aduncus$ ) fetus from Australia.

A late-term fetus was born dead to a mature captive-born Indo-Pacific bottlenose dolphin in a large open-water enclosure and was retrieved after 3 days. The fetus weighed 10 kg (normal size is approximately 30 kg), and samples of brain, heart, lung, liver, kidney, lymph node, and gastrointestinal tract were collected during necropsy and preserved in buffered neutral 10% formalin for histological assessment. Paraffin-embedded sections were cut at 5  $\mu m$  thickness, stained with hematoxylin and eosin (H&E), and examined microscopically. Selected sections were immunohistochemically stained with antibodies to *T. gondii* (Lindsay and Dubey, 1989) and *S. neurona* (Dubey and Hamir, 2000) as previously described.

Multiple tissues of the dolphin showed marked postmortem changes, including prominent invasion of the tissues with putrefactive bacteria. Important histological findings were recognized within the heart and the brain. The myocardium had multiple small areas of necrosis characterized by myofiber fragmentation and lysis with some mild nonsuppurative inflammation (Fig. 1). Protozoa were seen within the cells of the surrounding tissues. Some mild interstitial inflammation was evident in the epicardium. Similar protozoa were randomly distributed within the brain and were accompanied by a multifocal, nonsuppurative necrotizing encephalitis. No other important changes could be identified in the other tissues because of severe autolysis.

Tissue cysts and tachyzoites were both identified in close association with lesions (Fig. 1). Tissue cysts were often at the periphery of the lesion. The organisms stained strongly positive with anti-*T. gondii* antibodies and more organisms were seen in these sections than in those stained with H&E (compare Fig. 1A, B).

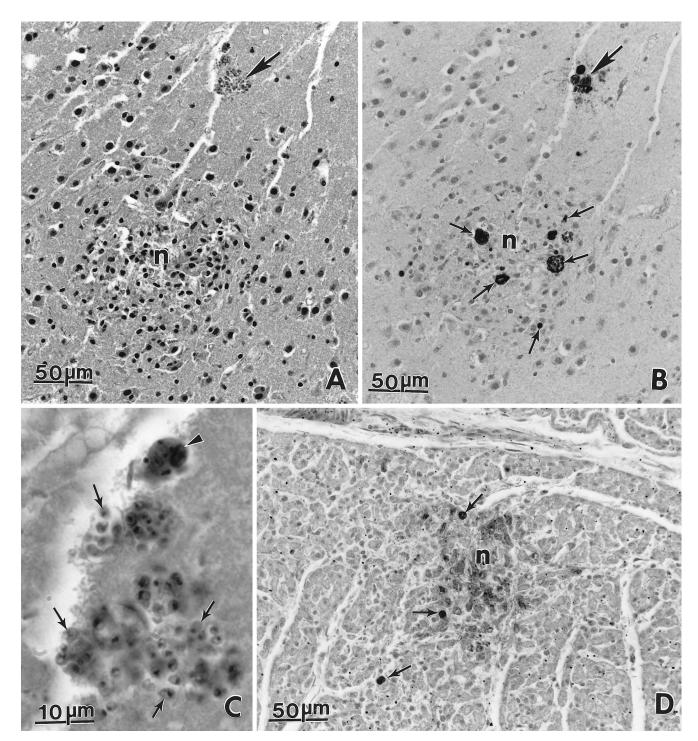


FIGURE 1. Lesions and *Toxoplasma gondii* in the brain (A–C) and the heart (**D**) of the dolphin fetus. **A, B.** Focal lesion of necrosis (n), inflammation, and a group of protozoa (arrows). **C.** Higher magnification of the *T. gondii* (arrow) in Figure 1A. Note partly degenerated tachyzoites (arrows) and a small tissue cyst (arrowhead). Bacillary rods are also present. **D.** Focal necrosis (n) and groups of *T. gondii* (arrows). H&E (**A, C**) and immunohistochemical stain with anti-*T. gondii* antibodies (**B, D**).

Although toxoplasmosis has been described in several species of marine animals, this is the first report of congenital toxoplasmosis in a marine animal. The dam of the fetus lived in a netted enclosure within a public marina and probably acquired the *T. gondii* infection by ingestion of food or water contaminated with oocysts. Feral cats frequent the quayside area.

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